Chapter

Adipose Tissue Remodeling during Cancer Cachexia

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Abstract

Cancer-induced cachexia (CC), characterized by systemic inflammation, body weight loss, adipose tissue (AT) remodeling, and muscle wasting, is a malignant metabolic syndrome with an undefined etiology. There is a consensus that multiple factors contribute to cancer-induced AT remodeling, and longitudinal studies show that patients lose AT before they start losing muscle mass. In CC, AT remodeling occurs predominantly through adipocyte atrophy, impairment of fatty acid turnover, inflammation, rearrangement of extracellular matrix (ECM), and browning of AT. More recently, some studies have shown that AT is affected early in the course of cachexia. Additionally, studies using experimental models have consistently indicated that the alterations in adipocyte metabolism begin quite early, followed by the downregulation of adipogenic and thermogenic genes. These sets of changes, in addition to metabolites derived from this process, maybe the initial (sterile) trigger of the sequence of events that result in the remodeling and dysfunction of AT in cachexia. Therefore, the present chapter aims to describe state of the art related to the subject of interest by analyzing the primary studies that have addressed the possible interface between inflammation and morphofunctional alterations of AT, in addition to the possible repercussions of this process during the development of CC.

Keywords: adipose tissue, cachexia, inflammation, metabolism, adipogenesis, ECM, browning

1. Introduction

1.1 Etiology of cachexia syndrome and adipose tissue remodeling

Cachexia is a common occurrence in the advanced stages of cancer and contributes to reduce the quality of life and life expectancy of patients [1, 2]. Cancer-related cachexia (CC) is a significant cause of morbidity and mortality, affecting more than 80% of individuals with advanced cancer and accounting for more than 20% of deaths [3–5]. Notably, the severity of cachexia does not correlate with tumor size [6]. Although there are descriptions of cachectic individuals dating back to more than 2000 years ago, increased attention has been focused on this syndrome in the recent decades. Nevertheless, its etiology remains unknown, and there is no treatment that is able to revert this condition [7, 8].

Cachexia is understood as a complex metabolic syndrome associated with underlying diseases and is characterized by a reduction of muscle mass and

a depletion of fat stores [9–11]. Thus, the main clinical symptoms of CC are body weight loss in adults (corrected for water retention) and impaired (substandard) growth in children (after exclusion of endocrine disorders) [12]. Anorexia, inflammation, insulin resistance, and increased degradation of muscle proteins are frequently associated with cachexia [1, 2]. Although anorexia exhibits different characteristics when compared with starvation, muscle mass loss due to aging (sarcopenia), primary depression, malnutrition, and hyperthyroidism is correlated with increased morbidity associated with asthenia and metabolic disorders [3, 13].

Weight loss is the foremost independent predictor of mortality in cancer patients [8, 12, 14], beginning with the loss of both a fat mass (adipose tissue—AT) and the lean body mass (skeletal muscle tissue). Over the last few years, the former has often been proposed to proceed more rapidly in the patient than the latter [11, 15, 16]. By the late 1980s and early 1990s, cachexia was being approached from a different perspective, leading to a new conception according to which it is considered a chronic inflammatory syndrome. It is currently believed that factors produced by both the tumor and the host cause anorexia and the metabolic abnormalities that result in cachexia [3, 17, 18]. Based on the knowledge obtained regarding cachexia and its complexity, the latest international consensus defined standard diagnostic criteria for the disease [1]. According to this consensus, the condition is categorized as pre-cachexia (early stage), cachexia, or refractory cachexia (late stage) based on the severity of the following parameters: (1) the reduction of total body mass; (2) the presence of metabolic disorders; (3) anorexia; and (4) systemic inflammation, as illustrated in **Figure 1A**.

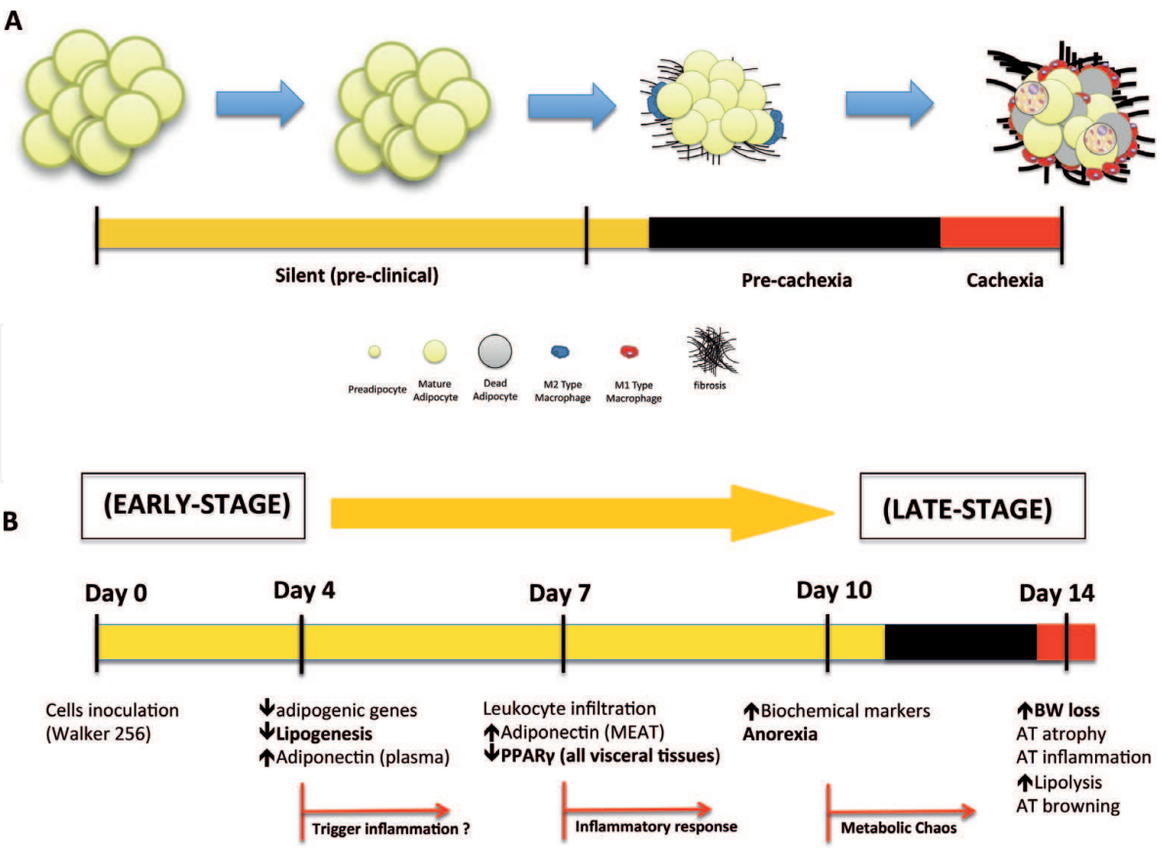

Figure 1.
Model of cachexia development from a translational point of view. (A) Morphofunctional changes in adipose tissue described in cancer cachexia patients. These alterations are associated with the stages of syndrome development, according to [1]. (B) Compilation of main metabolic and inflammatory changes described in the experimental model of cachexia induced by Walker 256 tumor.

An accurate understanding of the fundamental mechanisms that underlie CC is essential for the development of new pharmacological and nutritional therapies. In this way, several studies [19–23] have suggested that AT is the target of local and systemic factors derived from the host and by the tumor, including pro-cachectic factors [tumor necrosis factor α (TNF-α); interleukin (IL) 6; IL-1ß; IL-8; interferon-γ (INF-γ); ciliary neurotrophic factor; and leukemia inhibitory factor], anti-cachectic factors [soluble TNF-α receptor (sTNFR); soluble IL-6 receptor (sIL-6R); IL-1 receptor antagonist (IL-1RA); IL-4; IL-10; and IL-15], and tumor products [proteolysis-inducing factor (PIF); lipid-mobilizing factor (LMF); zinc-α 2-glycoprotein (ZAG); toxohormone-L; and anemia-inducing substance (AIS)]. Such factors are involved in the etiology and progression of cachexia [8, 14, 24–29]. Upon positing the inflammatory model as the hypothesis to be tested, some studies have recently demonstrated the relevance of subcutaneous adipose tissue (scAT) as both an important source of inflammatory mediators (particularly IL-6 and adiponectin) and an important source of biomarkers for cachexia (clinical progression), as adiponectin expression exhibits a correlation with the magnitude of the total body mass reduction [20]. More recently, a consistent modification consisting of inflammatory cell presence and fibrosis in scAT induced by cachexia was demonstrated in gastrointestinal cancer patients [30, 31]. The fibrosis was characterized by the presence of "crown-like structures" composed of CD68 positive AT macrophages (ATMϕs) surrounding adipocytes, and increased CD3 Ly, both of which were more evident in the fibrotic areas. In addition, some of these changes were already present in the cancer group, suggesting that AT inflammation may occur at an early stage of cachexia, even before the detection of pre-cachexic clinical features. Thus, alterations in AT inflammation seem to play a crucial role in the changes resulting in fat mass reduction, in addition to other morphofunctional alterations related to this tissue [19, 21]. Moreover, these changes appear to start quite early, long before any local tissue and/or systemic (circulatory system) changes can be detected.

Nevertheless, most studies on this topic have been restricted to assessing inflammation from the systemic point of view, only investigating plasma parameters, while neglecting tissue inflammation and, particularly, the events that precede the appearance of these alterations, although they may significantly contribute to disorders that result in AT remodeling, such as impairment of lipid metabolism, tissue cells turnover and inflammation, fibrosis, and subsequent systemic inflammation [2, 28]. Additionally, considering the important relationship among infiltrating cells (inflammatory mediators), the regulation of adipocyte metabolism, and the consequent remodeling of the AT, few or no studies have investigated this relationship and its role in the various stages of cachexia to our knowledge.

1.2 General features of adipose tissue remodeling

In general, AT remodeling in CC comprises a set of morphostructural modifications characterized by adipocyte atrophy, a result of an unbalance of lipids turnover, main due to increased lipolysis [10, 32]; impairment of adipocyte cellular turnover (adipogenesis) [18, 19]; enhanced inflammatory signaling [21, 30, 32]; modification of extracellular matrix (ECM) component generally resulting in fibrosis [30, 31]; and browning phenotype related to increase thermogenic effect [16, 33–35].

1.2.1 Adipose tissue atrophy is involved in the etiology of cachexia

The basic structure of the various types of AT includes mature adipocytes, stromal vascular cells (mesenchymal precursor cells, preadipocytes, fibroblasts, and immune system cells), blood vessels, lymph nodes, and nerves [36]. Fat cells

(adipocytes) are the main cell type in AT, while the presence of other cell types varies as a function of the tissue location [mesenteric (meAT), epididymal (epiAT), retroperitoneal (rpAT), or scAT], animal species, and disease (e.g., obesity and cachexia) [37]. Additionally, the importance of AT in the control of adiposity is well established, as is the role of the adipokines (leptin, adiponectin, and resistin, among others) released by the adipose tissue [38]. While alterations in the development and metabolism of adipocytes have been implicated in the pathogenesis of human immunodeficiency virus (HIV)-related lipodystrophy [39], very little is known about the molecular mechanisms involved in the occurrence of lipodystrophy associated with other conditions, such as cachexia.

As mentioned above, in the course of cachexia, the observed body weight loss predominantly results from a reduction in the fat (AT) and lean (skeletal muscle) mass [10, 40]. More recently, AT loss was found to precede the reduction of the lean body mass [11, 15], and thus, a more accurate understanding of this process began to emerge. Several factors have been suggested as the cause of the changes that lead to a reduction of AT mass, including (1)increased lipid mobilization due to increased lipolysis in adipocytes [9, 10]; (2) reduced lipogenesis, resulting from decreased lipoprotein lipase (LPL) activity [17]; and (3) impaired adipocyte turnover, most likely as a function of an adipogenesis-apoptosis imbalance in AT [41].

Taking the factors that are most likely to be involved in the observed fat mass loss and the relevance of AT in cachexia into consideration, the recent studies have utilized an experimental animal model that allows temporal assessment of the main variables that are potentially involved in cachexia-induced AT disorders, with an emphasis on the parameters related to adipogenesis, metabolism, and inflammation [18, 23, 27, 32], as shown in **Figure 1A**. These data demonstrate that these alterations start early, at 4 days after cachexia induction by inoculation of Walker 256 tumor cells in Wistar rats [19, 32]. It is worth noting that this is the period that precedes the appearance of the classic symptoms of cachexia, such as dyslipidemia, and a reduction of the total body weight, as well as inflammatory alterations in the AT in these animals, which begin to predominate starting on Day 7. In Lewis lung carcinoma (LLC) tumor-bearing mice, upregulation of genes related to lipid turnover and adipose browning is evident even before the detection of the body weight loss of the animals [16, 33]. Similarly, using several experimental models of cachexia (syngeneic and genetic), K5-SOS mice showed reduction in fat mass and spleen enlargement in the pre-cachexic period, that is, before the detection of body weight loss and the development of cachexia [33]. Taking the aforementioned findings into consideration, the temporal characterization of some of the alterations that occur in different AT depots in the course of cachexia has effectively pointed to actual pathways and mediators that might be involved in the earliest changes, in addition to serving as biomarkers for the clinical progression of cachexia.

As mentioned above, accentuated reduction of fat mass is a significant clinical sign of CC. Although it has not yet been well established, these alterations depend on the location of the adipose tissue involved (e.g., visceral or subcutaneous). Taking this into account, it was recently shown that, considering results found in the experimental animal model of cachexia (Walker 256 tumors cells-induced), visceral fat depots were affected in different ways. Thus, epiAT and meAT [25] exhibited higher reductions in relative weight (percentage of total body mass), respectively, while rpAT did not show any change [42]. Furthermore, in cachectic patients, assessment of the adipose tissue area by means of computed tomography in humans showed that visceral AT was reduced in cachectic individuals with gastrointestinal carcinosarcoma when compared with controls [43]. On the other hand, in individuals with gastrointestinal cancer, scAT seems to be more affected

when compared with visceral AT (epiAT and meAT), at least considering tissue inflammation parameters [44].

In fact, as described above, AT atrophy is a well-characterized clinical variable in cachexia syndrome. In addition, this tissue is affected early before the appearance of classic signs of cachexia. In this sense, the main morphological alteration observed as a result of AT atrophy is the alterations in the area and perimeter of adipocytes in both animal models [18, 42] and CC patients [20, 30]. Still in this context, depot-specific changes in adipocyte ultrastructure [42] were also described.

1.2.2 Metabolic features of adipocytes in the course of cachexia

The body weight loss in CC has been thought to be a result of profound changes in metabolic pathways of tissues and organs, which cannot be solely explained by enhanced energy expenditure or malnutrition [45]. In this regard, the role of early AT dysfunction seems to have gained importance in the onset and progression of many alterations induced by the syndrome. Different mechanistic possibilities have been proposed to explain the changes in AT in cachexia, such as increased lipolytic activity, decreased the activity of LPL, reduced de novo lipogenesis, and, consequently, decreased lipid triacylglycerol (TG) deposition [32, 45–47]. Adipocyte lipid turnover [i.e., the balance between incorporation and removal of TG into adipocytes, in which lipolysis (hydrolysis of intracellular TGs)] and is the most important factor for lipid removal [48]. In CC, human and animal models [11] have shown an increased rate of lipid mobilization, and longitudinal studies have shown that patients with CC lose AT mass before wasting of the muscle mass can be detected [15]. In addition, an accelerated rate of AT loss is believed to be associated with shorter survival time during cancer progression [49].

Most of the volume (>90%) of a white adipocyte is represented by a single fat droplet, which is mainly composed of TG. During periods of stress and/or nutrient deprivation, such as in metabolic disorders, the adipocytes activate mechanisms that lead to lipolysis, with a consequent release of non-esterified fatty acids (NEFAs) and glycerol originating in the TG stored in these cells. NEFAs are immediately released into the bloodstream and subsequently serve as a substrate for energy production of muscle tissues, or alternatively, they are taken up into the liver, where they are oxidized, esterified, or transformed into ketone bodies [50]. The reactions that result in NEFA release are mainly catalyzed by two enzymes: adipocyte triglyceride lipase (ATGL), which catalyzes the first step of the pathway, resulting in the formation of diacylglycerol, and hormone-sensitive lipase (HSL), which is responsible for additional hydrolysis and catalyzes the reactions that culminate in NEFA and glycerol release [10].

Among the mechanisms that may be involved in the metabolic disorders that cause fat mass reduction in cachexia, increased lipolysis appears to be the most evident and is being described as an increasing frequency [9, 11]. Das and colleagues found that in ATGL and HSL knockout mice, there was a greater resistance to the development of tumor-induced cachexia, which was more evident in ATGL-deficient animals. These authors also observed a positive correlation between ATGL activity and the severity of cachexia. Even more interestingly, they only detected a reduction of lean body mass in animals that exhibited severe cachexia. This phenomenon followed the events that led to a reduction of fat body mass, most noticeably involving an increase of TG hydrolysis in AT. The results of this study corroborate findings previously reported in individuals with cancer-related cachexia [15], thus increasing the consistency of the evidence demonstrating the role of AT as a target tissue in cachexia. Nevertheless, neither the exact time when

these changes occur in the course of cachexia nor the various affected depots have yet been adequately described.

To elucidate these aspects, a study was conducted in which the lipolytic capacity of isolated adipocytes was assessed at 4 and 14 days after the inoculation of tumor cells. Two particular visceral fat stores were selected (meAT and rpAT) based on the results of previous studies that demonstrated their relevance for the development of cachexia. Day 4 was selected to perform the analysis, also based on previous results [19], which demonstrated downregulation of the genes involved in adipocyte metabolism, while no changes were found in the assessed morphological and inflammatory parameters. Interestingly, the tumor-bearing animals exhibited a reduction in basal lipolysis 4 days after inoculation, while on Day 14, the cachectic animals exhibited a considerable increase in the basal lipolysis rate (**Figure 1B**). These findings corroborate with the results of other studies showing increased lipolysis in the subcutaneous AT of cachectic patients [9, 51].

In this regard, another important aspect was a deregulation of lipolysis (in vitro) revealed a distinct profile, depending on the degree of disease progression. In the first, the basal rate of lipolysis was reduced and was accompanied by increased p-HSL (Ser565) expression, which is regulated by AMPK activation and, consequently, inhibits HSL activity. Patients with CC show reduced spontaneous basal lipolysis with elevated *ex vivo* responses to catecholamine and natriuretic peptides [40, 52]. In this aspect, an elegant study showed that the lack of AMPK activity is a common feature of adipose tissue dysfunction in cachectic mice and is triggered, at least partially, through the aberrant induction of *Cidea* and the subsequent degradation of AMPK in this tissue [53]. The authors suggest that treatment of cachectic animals with a peptide specifically targeting the white adipose tissue AMPK-CIDEA interaction prevents AT loss under CC conditions.

1.2.3 Downregulation of adipogenic genes in cancer cachexia

Adipogenesis may be defined as the process of the differentiation of precursor cells (preadipocytes) into new adipose cells (adipocytes) that are able to store TG and synthesize and secrete various proteins called adipokines [54]. In fact, impaired adipogenesis might contribute to the development of obesity-related metabolic disorders, such as peripheral insulin resistance, hyperlipidemia, and type 2 diabetes [55, 56]. The process of adipocyte differentiation involves the activation of a cascade of transcription factors that coordinate the expression of genes that are responsible for adipocyte function [55, 57]. The initial events include transient increases in CCAAT/enhancer-binding proteins beta and delta (C/EBPβ and δ), which allow preadipocytes to be distinguished from non-adipogenic precursor cells and subsequently activate the expression of the peroxisome proliferator-activated receptor gamma-2 (*Pparγ-2*), which in turn stimulates the expression of *C/ebpα*, which exerts a synergic effect with PPARγ-2 on the control of terminal differentiation [54, 57]. Local and endocrine factors might regulate adipogenesis through the modulation of these transcriptional events [58].

Adipocyte maturation is accompanied by intracellular lipid accumulation, which is mainly mediated by sterol regulatory element-binding protein-1C (SREBP-1C). In addition to activating the expression of *Pparγ*-2, SREBP-1C also activates the lipogenic pathway by stimulating the expression of the genes that encode the main enzymes of that pathway, such as ATP-citrate lyase (*Acly*), acetyl-CoA carboxylase (*Acaca*), fatty acid synthase (*Fasn*), and stearoyl-CoA desaturase-1 (*Scd-1*) [18, 57]. In addition to its direct participation in adipocyte differentiation, PPARγ-2 also plays an important role in the transcriptional regulation of genes associated with the lipogenic pathway by inducing the transcription of genes that encode adipocyte

fatty acid-binding protein (*aP2*), LPL, fatty acid transport proteins (FATPs), and fatty acid-binding proteins (FABPs), among others. Activation of genes associated with glucose transport, such as *Glut*4, and thermogenesis, such as the mitochondrial uncoupling proteins (*Ucp2* and *Ucp3*), has also been described [18].

In AT, a balance between the growth/differentiation (adipogenesis) and death of its cells (generally by apoptosis) regulates the cellular turnover [41, 59]. In this aspect, the impairment of adipogenesis in the course of cachexia has been recently addressed. Some studies have elucidated the adipogenic marker profile during the development of cachexia syndrome [19, 25]. However, few studies have addressed the apoptotic processes and/or AT turnover during cachexia [43]. It has been known that adipogenic genes are downregulated in CC in epiAT [18] and rpAT [19]. On the other hand, scAT apoptosis did not change in cancer patients [43]. Therefore, considering that AT depots respond heterogeneously to CC and several metabolic and inflammatory pathways are involved in AT remodeling, there is no consensus if such effects induced by cachexia would be a result of secreted products directly by the tumor and tumor-host relationship. Thus, a recent study has analyzed in vitro adipogenesis in a co-culture system to mimic the effects of CC on adipocytes [60]. Co-culture of LLC cells promoted a decreased volume of the lipid droplets in 3 T3-L1 cells, compromising its maturation process (adipogenesis) in vitro. This result was followed by the downregulation of adipogenic and lipolytic gene expression, increased in apoptosis markers and proinflammatory cytokines secretion by both tumor cells and adipocytes. In this sense, these data suggest that the presence of the tumor cells was able to inhibit the adipocytes' maturation, which was associated with the increased levels of inflammatory cytokines.

In this way, the findings generated in the experimental model have demonstrated to be adequate for investigating cachexia-induced alterations of adipogenesis and point to the need to widen the scope of the assessed genes as well as the pathways and regulatory factors involved. Besides, modifications in adipogenesis appear to precede the appearance of the classic signs of cachexia as well as the signs of tissue inflammation in AT, such as increases in infiltrated macrophages and the production of inflammatory cytokines [21]. Thus, the factors that "silence" the genes involved in the differentiation of preadipocytes, and, consequently, in the maintenance of adipose cells turnover in AT might play a central role in the genesis of the damaging changes (metabolism and function) that occur in the adipose tissue of individuals with cachexia.

1.2.4 Adipose tissue inflammatory profile in the course of cachexia

According to the abovementioned findings from animal models and cancer cachexia patients, a metabolic and morphological dysfunction that results in the AT remodeling occurs during the development of CC [2]. More recently, another relevant aspect of cachexia-induced AT remodeling is the establishment of AT inflammation, which is characterized by increased recruitment of ATMϕs, including activated M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages [32]. In this way, an inflammatory profile predominates the end-stage of cachexia, particularly in visceral AT, as most of the characteristic changes of this syndrome are already established at this time [19, 27]. More recently, the immune cell infiltration profile was analyzed in greater detail, which was found to be characterized by an increase in M1-polarized macrophages [20]. In that study, the profile of chemokines specific to polymorphonuclear cells was also investigated, in addition to the presence of neutrophils in the various AT depots. The results revealed an increase in the chemokines CCL3 and CXCL2 at 7 days after tumor cell inoculation. The presence

of CD11b-positive cells, which were tested to detect the presence of neutrophils, was observed in the same period. Taking the temporal changes identified in the cachexia model into consideration, as a function of the assessed parameters, the results indicate that inflammation starts on Day 7 and is established by Day 14, a period during which a series of disorders characteristic of cachexia become evident (e.g., a reduction of the total body and fat mass, dyslipidemia, and hypoglycemia). In this regard, even more recently, these findings were presented in greater detail in cachexia induced by LLC cells, showing ATMϕs polarization tends to be directed to M1 phenotype [61, 62].

Despite the increasing perception of the importance of the relationship between inflammation and CC, and systemic inflammation in particular, there is still no consensus regarding its source and also the role of inflammation of TA in the establishment and development of cachexia, among cancer patients in particular [9, 43]. Limitations in experimental designs, the selection of control groups, and the techniques used to analyze markers of inflammation have most likely been responsible for preventing a more precise investigation of the presence of inflammation in AT. Addressing this question, a study has recently demonstrated increased gene expression of phenotypic markers of ATMϕs and inflammatory cytokines, such as IL-6 and TNF-α, in cachectic patients with gastrointestinal cancer [20]. Interestingly, increased gene levels of IL-6 were positively correlated to increase plasmatic levels of this cytokine, indicating that in cachectic patients, scAT may be an important source of inflammatory mediators. Even more recently, the same group revealed an increase in ATMϕs forming crown-like structures in the same AT depot from cachectic patients [44], which is a characteristic finding in fat tissue in experimental animal models of obesity and in obese patients [63]. In addition, an increase in chemoattractant for ATMϕs gene expression in scAT, such as *Ccl2*, was detected only in cancer patients without cachexia, showing no changes in cachectic ones. However, despite the relevance of local inflammation, in AT in particular, the mechanisms responsible for both cachexia and inflammation remain to be elucidated. The characterization and understanding of the process of inflammation in cachexia are also relevant to establish whether it is secondary to or the "trigger" for the development of cachexia syndrome.

1.2.5 Alterations in the extracellular matrix of adipose tissue

Extracellular matrix (ECM) remodeling is the result of the processes of matrix synthesis and degradation during which specific proteins are deposited, such as tenascin and fibrin, and occurs under both physiological (e.g., tissue repair) and pathological conditions (e.g., inflammation) [64]. The ECM consists of a complex network of multifunctional and structural molecules, including various collagen isoforms, adhesive glycoproteins, and proteoglycans. This network provides support to cells and to the signaling pathways that control their migration, proliferation, and differentiation. Also, the ECM might serve as a reservoir of cytokines and other growth factors, which are released into the system in variable amounts depending on the pathological condition.

ECM remodeling plays a central role in the differentiation of adipocytes. Although the corresponding molecular mechanisms are only partially understood, ECM remodeling occurs concomitantly with the activation and/or repression of a transcriptional "network" involved in adipogenesis, which may be activated or repressed due to extracellular stimuli [65]. In the 3 T3-L1 mouse cell line, the differentiation of adipocytes is associated with a reduction in the fibronectin-rich matrix and basal lamina formation [66, 67]. Silencing of the pericellular collagen

membrane type-1 matrix metalloproteinase (*Mt1-Mmp*) gene in the course of the mouse development results in the formation of a rigid collagen fibril chain and changes *in vivo* adipogenesis [68]. This condition is an example of the relationship between structural changes in ECM and adipocyte differentiation.

ECM remodeling plays a central role in the differentiation of adipocytes. Although the similar molecular mechanisms are only partially understood, ECM remodeling occurs concomitantly with the activation and/or repression of a transcriptional "network" involved in adipogenesis, which may be activated or repressed due to extracellular stimuli [69]. While some such alterations, such as changes in collagen content deposition, an increase in the number of infiltrated cells and insulin resistance, are also present in cancer-related cachexia, very little is known regarding the possible relationship between these cell types (e.g. fibroblasts, pre-adipocytes, immune cells, and others) and the processes that lead to ECM remodeling.

In MAC16 tumor-bearing mice, a cancer cachexia animal model, shrunken adipocytes and increased collagen-fibril content in AT were reported [18]. In CC patients, our group recently showed that the total type I collagen content of the scAT is rearranged in cachectic individuals with gastrointestinal cancer, which is associated with an increase in macrophage and lymphocyte contents. Interestingly, the total collagen content exhibited discrete changes in cancer patients without cachexia, but the expression of the *Ccl2* gene was found to be increased [20]. Another exciting finding demonstrates that ECM remodeling of AT in cancer cachexia results in augmented collagen fiber content. Excessive synthesis of mature elastic fibers accompanies such morphological scenery, besides strong labeling for collagen type I (COL1) and III (COL3) in the AT from cachectic patients [30, 31]. Besides, the presence of fibrosis was also associated with an increased number of myofibroblasts and an activated TGFβ/SMAD pathway in the subcutaneous AT of gastrointestinal cancer cachectic patients [31, 70].

These findings indicate that the morphological changes that lead to AT remodeling in CC patients are evident, albeit discretely, before the onset of the earliest characteristic symptoms of cachexia (**Figure 1**). However, to the best of our knowledge, no study has yet investigated the causes and repercussions of fat remodeling in the course of cachexia in full detail.

1.2.6 Browning of adipose tissue induced by cancer cachexia

Within the set of morphofunctional changes that result in the AT remodeling, it has recently been shown that cachexia induces browning of AT in addition to changes in immune-modulatory activity. In this scenario, chronic inflammation and β-adrenergic activation of thermogenesis functionally cooperate in the pathogenesis of cachexia [16, 33, 62, 71]. In general, browning of AT has been described as responsible for the increase in total caloric expenditure [72], and the induction of browning has therapeutic potential in promoting the reduction of body fat [16, 33, 34, 73]. However, this fact refers to conditions of diseases characterized by the presence of metabolic disorders, usually associated with high caloric intake, overweight, and/or obesity [74].

In this sense, in CC, the presence of the browning phenotype has shown to be detected very early [16] in different experimental models [33, 34, 35]. Also, it has demonstrated a significant role in altering the metabolism of this tissue because this process is related to the increase in energy expenditure and mobilization of fatty acids [17] by adipocytes. Another interesting new fact was that, in this same study, AT from cachectic mice showed upregulation of particular genes for brown

adipocytes when compared to samples of brown adipose tissue. This fact is not usual because the one would expect an increase in genes specific to beige adipocytes. Also, regarding thermogenesis, the rectal temperature was reduced when the main clinical signs of the diseases were already established (refractory cachexia). Interestingly, this hypothermic phenotype has previously been described in the Walker 256 tumor-induced cachexia model, in the final stage of cachexia [75]. On the other hand, additional studies are needed to clarify the immuno-metabolic changes resulting in thermogenic adjustments induced by the syndrome, as well as the particular clinical consequences.

In this same study [33], the browning phenotype has also been described in samples of visceral adipose tissue from cancer cachexia patients. However, there is a need for analysis in a larger cohort and additional characterizations about the possible physiological repercussions for these patients. Also, there is still a need to characterize the real contribution of AT browning to overall energy expenditure during cancer cachexia. In this sense, an elegant study has evaluated, in several experimental models, that although the studies above detected mild induction of *Ucp1* mRNA levels in tumor-exposed AT, such changes appear to be discrete in thermogenic terms [53]. In this scenario, the overall effect of AT UCP1-dependent thermogenesis on systemic energy homeostasis may not be the principal actor during cancer cachexia.

2. Concluding remarks

In summary, several studies have shown that AT is significantly affected during the development of cachexia. The main alterations related to metabolic disorders, particularly those involving early adipocyte lipid turnover dysfunction of AT, increases in immune cell infiltration followed by increased local production of inflammatory mediators and remodeling of ECM components. More recently, some studies have shown that cachexia-induced browning of AT is a characteristic phenotype that arises from alterations that result in the AT remodeling, although its function is still not well characterized. Nevertheless, studies using those experimental models have consistently indicated that the modifications in the adipocyte metabolism begin quite early, and the metabolites derived from this process may be the initial (sterile) trigger of the sequence of events that result in the remodeling and consequent dysfunction of AT in cachexia. Finally, a deeper understanding of the initial stimulus that triggers AT dysfunction, in particular, inflammation and remodeling, needs to be further studied because evidence indicates that AT dysfunction plays a significant role in cachexia and may be a potential modulator of the process that could be explored therapeutically.

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Conflict of interest

The authors declare no conflicts of interest.

Appendices and nomenclature

ECM	extracellular matrix
ATGL	adipose triglycerides lipase
HSL	hormone-sensitive lipase
CC	cancer-related cachexia
AT	adipose tissue
sTNFR	soluble tumor necrosis factor-α receptor
sIL-6R	soluble IL-6 receptor
IL-1RA	IL-1 receptor antagonist
IL	interleukin
PIF	proteolysis-inducing factor
LMF	lipid-mobilizing factor
ZAG	zinc-α2-glycoprotein
AIS	anemia-inducing substance
scAT	subcutaneous adipose tissue
CD	cluster of differentiation
ATMϕs	adipose tissue macrophage
CD3	cluster of differentiation 3
Ly	lymphocytes
HIV	human immunodeficiency virus
LPL	lipoprotein lipase
LLC	Lewis lung carcinoma
K5-SOS-F	keratinocyte-specific expression of an HA tagged dominant form of the human SOS1
TG	triacylglycerol
NEFAs	non-esterified fatty acids AMPK
AMPK	5′ adenosine monophosphate-activated protein
CIDEA	cell death-inducing DFFA-like effector a
C/EBP	CCAAT/enhancer-binding proteins
PPARγ	peroxisome proliferator-activated receptor gamma
SREBP-1C	sterol regulatory element-binding protein-1C
ACC	acetyl-CoA carboxylase
FAS	fatty acid synthase
SCD-1	stearoyl-CoA desaturase-1
GPAT	glycerol-3-phosphate acetyltransferase
aP2	adipocyte fatty acid-binding protein
FATPs	fatty acid transport proteins
FABPs	fatty acid-binding proteins
GLUT-4	glucose transport 4
UCP	mitochondrial uncoupling proteins
3 T3-L1	embryo fibroblast cells with a continuous substrain (L1) of 3T3 (Swiss albino) developed through clonal isolation
M1	polarized macrophages 1
M2	polarized macrophages 2
CCL	chemokine (C-C motif) ligand
CXCL	chemokine (C-X-C motif) ligand

MT1-MMP	collagen membrane type-1 matrix metalloproteinase
MAC16	murine adenocarcinoma 16
CCR2 (MCP-1)	C-C chemokine receptor type 2
COL1	collagen type I
COL3	collagen type III
TGFβ	transforming growth factor beta
SMAD	small worm phenotype mothers against decapentaplegic

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